FORM PTO-1390 (REV 5-93)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY DOCKET NO. P100564-00033
	TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	DATE: October 6, 2000
		U.S. APPLN. NO. (IF KNOWN, SEE 37 CFR 1.5) 09/646892

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/02171	30 March 1999	9 April 1998

TITLE OF INVENTION: T CELL RECEPTOR EPXRESSION CASSETTE

APPLICANT(S) FOR DO/EO/US: Petra JANTZER

1. XX This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
   (THE BASIC FILING FEE IS ATTACHED)

2. _ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. XX This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT articles 22 and 39(1).

4. XX A proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. XX A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. XX is transmitted herewith (required only if not transmitted by the International Bureau).
   b. XX has been transmitted by the International Bureau.
   c. _ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. XX A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. _ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. _ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. _ have been transmitted by the International Bureau.
   c. _ have not been made; however, the time limit for making such amendments has NOT expired.
   d. _ have not been made and will not be made.

8. _ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. _ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. _ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. XX An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. _ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. _ A FIRST preliminary amendment.
    _ A SECOND or SUBSEQUENT preliminary amendment.

14. _ A substitute specification.

15. _ A change of power of attorney and/or address letter.

16. XX Other items or information: PCT/RO/101, PCT/ISA/210, PCT/IPEA/409,
    CHECK NO. 302139
    8 Sheets of Formal Drawings

422 Rec'd PCT/PTO 06 OCT 2000

U.S. APPLN. NO. (IF KNOWN, SEE 37 C.F.R. 1.5) 09/646892	INTERNATIONAL APPLICATION NO. PCT/EP99/02171	ATTORNEY DOCKET NO. P100564-00033 DATE: October 6, 2000

17. XX The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)-(5):

Search Report has been prepared by the EPO or JPO.....$860.00
International preliminary examination fee paid to USPTO (37 CFR 1.492)....$690.00
No international preliminary examination fee paid to USPTO (37 CFR1.492) but international search fee paid to USPTO (37 CFR 1.492(a)(2))....$710.00
Neither international preliminary examination fee (37 CFR 1.492) or international search fee (37 CFR 1.492(a)(3)) paid to USPTO.....$1,000.00
International preliminary examination fee paid to USPTO (37 CFR 1.492) and all claims satisfied provisions of PCT Article 33(1)-(4) .....$ 100.00

	CALCULATIONS	PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860	
Surcharge of $130.00 for furnishing the oath or declaration later than _ 20 _ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	- 20 =		X $ 18.00	$00	
Independent Claims	- 3 =	00	X $ 80.00	$00	
Multiple dependent claim(s) (if applicable)			+ $270.00	$00	
TOTAL OF ABOVE CALCULATIONS =				$860	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$00	
SUBTOTAL =				$860	
Processing fee of $130.00 for furnishing the English translation later the _ 20 _ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$00	
TOTAL NATIONAL FEE =				$860	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$00	
TOTAL FEES ENCLOSED =				$860	
				Amount to be refunded	$
				Charged	$

a. XX A check in the amount of $860 to cover the above fees is enclosed.
b. _ Please charge my Deposit Account No. 01-2300 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.
c. XX The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-2300 .

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) and 1.27 (d) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION GSF-Forschungszentrum für Umwelt und Gesundheit GmbH
ADDRESS OF ORGANIZATION Ingolstädter Landstraße 1
~~85758 Oberschleissheim Germany~~
85764 Oberschleissheim, Germany

TYPE OF ORGANIZATION

[ ] UNIVERISITY OR OTHER INSTITUTION OF HIGHER EDUCATION
[ ] TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501 (c) (3))
[ ] NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA
(NAME OF STATE _____)
(CITATION OF STATUTE _____)
[XX] WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) AND 501(c) (3) IF LOCATED IN THE UNITED STATES OF AMERICA
[ ] WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
(NAME OF STATE _____)
(CITATION OF STATUTE _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled T cell receptor expression cassette by inventor(s) Schendel Dolores and Jantzer Petra described in

[ ] the specification filed herewith
[ ] application serial no. _____, filed _____
[ ] patent no. _____, issued _____

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention. If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invenion is listed below * and no rights to the invention are held by any person, other than the inventor, who could not qualify as small business concern under 37 CFR 1.9 (d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9(e). *
NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING × ppa. Stefan Reiserer i.V. Dr. Georg Menache
TITLE IN ORGANIZATION × Head of Legal Dept. Technologytransfer-Manager
ADDRESS OF PERSON SIGNING × Ingolstädter Landstr. 1, 85764 Oberschleissheim, Germany
SIGNATURE × ppa. [illegible] i.V. [illegible]

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

SCHENDEL et al

Serial No.: 09/646,892

Filed: October 6, 2000 Atty. Docket No.: 100564-00033

For: T CELL RECEPTOR EXPRESSION CASSETTE

PRELIMINARY AMENDMENT

Commissioner of Patents
Washington, D.C. 20231

November 15, 2000

Sir:

Prior to calculation of the filing fee and prior to the examination of this application, please amend the above-identified application as follows:

IN THE CLAIMS:

Claim 3, line 1, delete "or 2".

Claims 4, 5, and 7-12, lines 1 and 2 of each, delete "one of the preceding claims" and insert therefor --claim 1--.

Claim 16, lines 2 and 3, delete "one of claims 1 to 13" and insert therefor --claim 1--.

Claim 17, lines 2 and 3, delete "one of claims 13 to 14" and insert therefor --claim 13--.

Claim 19, line 1, delete "or 18".

Claim 20, line 1, delete "one of claims 17 to 19" and insert therefor --claim 17--.

Claim 21, line 1, delete "one of claims 17 to 20" and insert therefor --claim 17--.

Claim 22, line 1, delete "one of claims 17 to 21" and insert therefor --claim 17--.

Claim 23, line 4, delete "or 14".

Claim 24, lines 3 and 4, delete "one of claims 1 to 12" and insert therefor --claim 1--.

Claim 25, line 5, delete "one of claims 1 to 12" and insert therefor --claim 1--.

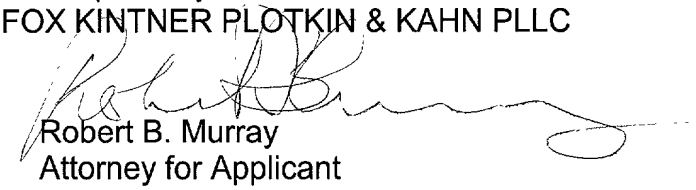
Claim 26, line 1, delete "or 25".

REMARKS

The above amendment to the claims has been made to correct the multiple dependency of the claims and to put the application in better condition for examination.

In the event that any fees are due in connection with this paper, please charge our Deposit Account No. 01-2300.

Respectfully submitted,
ARENT FOX KINTNER PLOTKIN & KAHN PLLC



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RBM/cb

T cell receptor expression cassette

Description

The invention relates to a process for expressing T cell receptors and to vectors which are suitable for this. In addition, this present document discloses cells which are transfected with the vectors and which are able to express T cell receptors which are in each case desired.

The T lymphocytes of the immune system are responsible for the cellular immune response. In this connection, diseased body cells or tumor cells are recognized by the so called T cell receptor (TCR), which binds an antigen which is specific for the diseased cell and which is in the form of short peptide fragments. These peptide fragments are presented at the cell surface by MHC molecules.

T cell receptors consist of two different polypeptide subunits, usually the so called T cell receptor α and β chains, which are linked to each other by way of a disulfide bridge. The α and β chains are in turn composed of variable and constant regions. The variable regions of the α chain comprise V and J gene segments while the variable regions of the β chain comprise V, D and J gene segments.

The chromosomal TCRα chain gene consists of approx. 50 to 60 variable segments, each of which contains an exon for a Vα gene segment, upstream of which is another exon which encodes a leader sequence which enables the protein to be imported into the endoplasmic reticulum and transported to the cell surface. A group of 61 J segments is located at a considerable distance from the Vα segments. The J segments are followed in turn by a single Cα segment for the constant region, which in turn contains separate exons for the constant region

and the hinge region and also an exon for the transmembrane and cytoplasmic regions.

The chromosomal TCRβ chain locus contains a group of approx. 65 Vβ gene segments which are located at some distance from two separate clusters which each contain a single Dβ segment and 6 and 7 Jβ segments, respectively, as well as a single Cβ segment. Each constant segment of the β chain possesses separate exons for the constant region, the hinge region, the transmembrane region and the cytoplasmic region.

During the development and maturation of the T cell, the separate gene segments are linked by means of somatic recombination. In the case of the α chain, a Vα gene segment comes to be located next to a Jα gene segment. This produces a functional exon. Transcription, and splicing of the VJα exon to the constant region, results in the formation of the mRNA which is translated into the TCRα chain. The rearrangement of the Vβ, Dβ and Jβ gene segments, which encode the variable domain of the β chain, creates a functional exon which is transcribed and added onto Cβ by means of splicing. The resulting mRNA is translated into the TCRβ chain. After their biosynthesis, the α and β chains join together to form the α:β TCR heterodimer. The hypervariable region of the TCR, which region is responsible for the specificity of the antigen recognition and is located in the region where the V, (D) and J gene segments are linked, is designated CDR3.

The limited availability of native T cells frequently places a substantial restriction on immunological investigations, at the functional and molecular levels, of the T cell receptors which are expressed by these cells. On the other hand, because of the above-described, complex assembly of the T cell receptor from two genes, which are in turn composed of a large number

of segments, it is no easy matter to prepare complete T cell receptors recombinantly in foreign cells.

The object underlying the present invention consequently consisted in making available a novel system for recombinantly expressing T cell receptors, which system allows defined MHC-restricted T cell receptors to be expressed and consequently enables them to be investigated at the functional and molecular levels.

This object is achieved by making available an expression unit which contains expression cassettes for TCRα chains and TCRβ chains, respectively. These expression cassettes in each case contain at least the 3′ segment of the constant moieties of the TCRα chain and β chain genes, respectively, with artificially inserted restriction cleavage sites, in particular multiple cloning sites possessing several restriction cleavage sites, with preferably at least one of the cleavage sites being a unique cleavage site, being present in the 5′ region of these segments. When several cleavage sites are present, the cleavage site which is located furthest 3′ is of particular importance since the cleavage sites which are located 5′ of it are lost when the V gene is cloned. As a result of exploiting the degeneracy of the genetic code, the introduction of these cleavage sites does not result in any amino acid substitution in the TCR chains. In order to complete the TCR chains, the variable TCR domains also have to be cloned upstream of the constant domains which are already contained in the basic vector. If required for a special V gene segment, the artificially inserted restriction cleavage sites can be inactivated using restriction endonucleases having a partially identical recognition sequence when the variable moiety is cloned.

The expression vectors according to the invention enable TCR sequences, in particular human TCR sequences, to be expressed in a simple manner in eukaryotic cells. Three advantages, in particular, are gained in this connection:

1. The expression cassettes can essentially be used for any TCR sequences and are therefore independent of the rearranged V region genes.
2. The DNA fragments which are to be cloned into the expression cassettes according to the invention, and which are PCR products, for example, are only short, which means that it is possible to simplify the control sequencing and/or minimize the error rate during the amplification. Preferably, therefore, the artificial restriction cleavage sites for cloning the V regions are if at all possible located in the 5′ region of the C regions.
3. The restriction cleavage sites which are inserted by mutagenesis do not result in any reading frame shift or any amino acid substitution in the final polypeptide. In addition, it is preferred that no identical endogenous cleavage sites should be located within the corresponding fragments, thereby making it possible to avoid a partial digestion during the cloning.

A first aspect of the present invention relates to a basic vector for preparing a TCR expression vector, which basic vector possesses an expression control sequence which is operatively linked to a polycistronic, preferably bicistronic, expression unit comprising:

(a) at least a part of the nucleotide sequence encoding a C region of the TCRα chain, with at least one restriction cleavage site being located in the 5′ region of the nucleotide sequence, and

(b) at least a part of the nucleotide sequence encoding a C region of the TCRβ chain, with at least one restriction cleavage site being located in the 5′ region of the nucleotide sequence.

The basic vector can be a prokaryotic or a eukaryotic vector. Preference is given to it being a vector which can be propagated in eukaryotic cells, in particular in mammalian cells such as human cells. Examples of eukaryotic vectors are described in Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd Edition (1998), Cold Spring Harbor Laboratory Press, Chapter 16, and Winnacker, Gene und Klone, "Eine Einführung in die Gentechnologie" [Genes and Clones, "An Introduction to Recombinant DNA Technology"] (1985), VCH Verlagsgesellschaft, in particular in Chapters 5, 8 and 10. The basic vector can be a chromosomal or episomal vector. Particular preference is given to the vector being a vector which can be replicated episomally, in particular a plasmid.

Apart from the polycistronic expression unit, the basic vector according to the invention contains additional sequenceelements which are customary for expression vectors, e.g. one or more selection marker genes, such as antibiotic resistance genes, one or more origins of replication and also the expression control sequence which is necessary for transcribing the expression cassette. This expression control sequence can be a prokaryotic or eukaryotic expression control sequence. Particular preference is given to an expression control sequence which is active in eukaryotic cells, in particular in mammalian cells. This expression control sequence contains a promoter and, where appropriate, transcription regulation and/or activation sequences, such as enhancers.

The polycistronic expression unit according to the invention contains two nucleotide sequences, one of

which encodes at least a part of the C region of a TCRα chain while the second encodes at least a part of the C region of a TCRβ chain. The C region is preferably a human Cα or Cβ region. Advantageously, these nucleotide sequences contain the complete 3′ region of the respective C regions. On the other hand, the presence of a complete 5′ region is not absolutely necessary since, as explained below, this can be cloned into the basic vector together with the respective V region at a later time. At least one restriction cleavage site is in each case located in the 5′ region of the nucleotide sequences encoding the C region. These restriction cleavage sites are preferably in each case different for the TCRα chain and for the TCRβ chain. Particularly preferably, they are unique restriction cleavage sites, i.e. restriction cleavage sites which only occur once in the entire vector. The introduction of these restriction cleavage sites may possibly result in a mutation in the nucleotide sequence encoding the C region. However, this mutation is preferably a "silent" mutation, i.e. a mutation which does not result in any amino acid substitution in the C region. Examples of suitable restriction cleavage sites which can be inserted into the 5′ region of the nucleotide sequence encoding the C region of the TCRα chain are BamHI and/or XmaI cleavage sites. These restriction sites can be introduced simply by effecting mutations in codons 5 and 6 of exon 1 of the C region of the TCRα chain (cf., e.g., Fig. 6). Particularly preferably, the cassette cleavage site is a BamHI site in codon 6 of the Cα gene. Examples of restriction cleavage sites for the C region of the TCRβ chain are SpeI and/or SalI cleavage sites which are introduced by mutations effected in codons 28 and 29 of the nucleotide sequence encoding the Cβ region (cf. Fig. 7). Particular preference is given to the cassette cleavage site being a SpeI site in codon 29 of the Cβ gene.

Particularly preferably, restriction cleavage sites are introduced into a region which encodes the first fifty amino acids of the respective C region of the TCR chain gene. Particularly preferably, the TCRα chain and TCRβ chain genes are genes of human origin whose sequences are described in Yoshikai et al., (Nature 316 (1985), 837-840) and Toyonaga et al., (Proc. Natl. Acad. Sci. USA 82 (1985), 8624-8628).

In addition, the expression unit of the basic vector according to the invention preferably contains a sequence which permits capping-independent translation of the polycistronic mRNA molecule which is produced by transcribing the expression unit. An example of such a sequence is an IRES sequence as occurs in a large number of different organisms, for example viruses. Thus, IRES sequences are present in the Picornaviridae, e.g. cardioviruses such as encephalomyocarditis virus, enteroviruses such as poliovirus, rhinoviruses such as human rhinovirus, hepatoviruses such as human hepatitis A virus (Fields et al., Virology, 3rd Edition (1996), Lipincott-Raven Publishers, Philadelphia), in Flaviridae, e.g. pestiviruses such as bovine viral diarrhea virus (Vassiler et al., J. Virol. 71 (1997), 471-478) or classical swine fever virus (Rijnbrand et al., J. Virol. 71 (1997), 451-457), retroviruses such as HTLV-1 (Attal et al., FEBS Lett. 392 (1996), 220-224) or Moloney murine leukemia virus (Vagner et al., J. Biol. Chem. 270 (1995), 20376-20383), Leishmania RNA virus 1 (Maga et al., Mol. Cell. Biol. 15 (1995), 4884-4889), or in humans, e.g. in the BiP gene (Yang and Sarnow, Nucleic Acids Res. 25 (1997), 2800-2807) or in the human fibroblast growth factor 2 gene (Vagner et al., Mol. Cell. Biol. 15 (1995), 35-44) or in Drosophila, e.g. in the E74A gene (Jones et al., Insect. Biochem. Mol. Biol. 24 (1994), 875-882). In order to enable expression of the TCRα chain gene and the TCRβ chain gene to be as high and as even as possible, the expression unit contains a ribosomal

translation initiation site, e.g. the so-called Kozak sequence, directly 5′ of each TCR chain sequence.

Preference is given to expression of the TCRα chain and of the TCRβ chain being as stoichiometric as possible. In addition to this, expression of the TCRα chain can also be limited in comparison to expression of the TCRβ chain. To achieve this, the expression cassette according to the invention can, for example, be structured in the 5′-3′ direction as follows: TCRβ chain gene; IRES sequence; TCRα chain gene. Alternatively, the ribosomal initiation sequence for the TCRα chain gene can, for example, be modified such that translation initiation is somewhat weaker than in the case of the TCRβ chain gene.

Another aspect of the present invention relates to a TCR expression vector which possesses an expression control sequence which is operatively linked to a polycistronic, preferably bicistronic, expression unit comprising:

(a) a nucleotide sequence encoding a complete TCRα chain
and
(b) a nucleotide sequence encoding a complete TCRβ chain,

with the nucleotide sequences encoding the V regions and C regions of the TCR chains being linked to each other by way of restriction cleavage sites in the 5′ region of the C regions. Preferably, the nucleotide sequences encoding the TCR chains possess at least one base substitution, as compared with the natural TCR sequence, in the region of the restriction cleavage sites, with the base substitutions being selected within the context of the degeneracy of the genetic code, i.e. leading to a "silent mutation".

In principle, the TCR expression vector according to the invention can be prepared by two different methods,

namely by firstly preparing a basic vector and inserting the nucleotide sequences encoding a desired V region into it, or by combining the nucleotide sequences which encode the desired V regions with the corresponding C regions in two separate vectors and then forming the expression vector from these two vectors. Another aspect of the present invention is consequently a process for preparing a TCR expression vector, comprising the steps of:

(a) preparing a basic vector as previously indicated, and

(b) inserting nucleotide sequences which contain the regions encoding a desired V region of a TCRα chain or TCRβ chain into the restriction cleavage sites which are located in the 5′ regions of the nucleotide sequences encoding the C regions, or

(a′) preparing two vectors, each of which contains at least a part of the nucleotide sequence encoding the C region of the TCRα chain or the TCRβ chain, respectively, with at least one restriction cleavage site being in each case located in the 5′ region of the nucleotide sequence,

(b′) inserting nucleotide sequences which contain the regions encoding a desired V region of a TCRα chain or a TCRβ chain into the restriction cleavage sites which are located in the 5′ regions of the nucleotide sequences encoding the C regions, and

(c′) assembling the nucleotide sequences encoding the TCR chains into an expression unit in order to obtain a TCR expression vector.

Yet another aspect of the present invention is a cell which is transformed with a basic vector or with a TCR expression vector as described above. The cell can be a prokaryotic cell, e.g. a Gram-negative bacterial cell, in particular E. coli. However, a cell used for TCR expression is preferably a eukaryotic cell, a mammalian cell and, in particular, a human cell. Examples of

methods for introducing the vectors according to the invention into such cells are to be found in Sambrook et al., loc. cit., and Winnacker, loc. cit.

Particular preference is given to using, for the transformation, a recipient cell which is able to express one or more accessory molecules, i.e. molecules which are required for exercising the T cell function. Examples of such accessory molecules are the cell surface markers CD3, CD4 and CD8 and cytokines such as IL-2 and/or TNF. The greatest preference is therefore given to the recipient cell being a human T cell. Examples of suitable human T cells are the T cell clones and lines such as 234 (Prof. Wank, Institut für Immunologie [Immunology Institute], LMU München, Goethestr. 31, 80336 Munich), Molt-4 (ATCC CRL 1582), Peer (Schlesinger et al., Thymus. 2 (1981), 235-243) and Jurkat (ATCC TIB-152) and variants thereof such as Jurkat 9-5 (Boehringer Mannheim GmbH). Particular preference is given to using cytotoxic T cells.

Yet another possibility for obtaining T cells which express a TCR having the desired specificity is that of introducing a TCR expression vector according to the invention into the germ line of an animal and isolating the T cells from the resulting transgenic animal or its progeny. Preference is given to preparing transgenic mice. In addition, preference is given to the transgenic mice also expressing accessory molecules, such as the human CD8 molecule and/or the human HLA-A*0201 molecule, as well as the TCR.

The invention consequently also relates to a process for expressing a TCR, with a suitable host cell being transformed with a TCR expression vector and the cell being cultured under conditions which lead to expression of a TCR, preferably to expression of the TCR as a membrane-bound TCR heterodimer.

Yet another part of the subject-matter of the present invention is a reagent kit for preparing a TCR expression vector. In a first embodiment, the reagent kit contains a basic vector, as previously defined, and primers for amplifying V regions of the TCRα and TCRβ chain genes. In a second embodiment, the reagent kit contains two separate vectors which separately contain the elements of the expression unit from the basic vector as well as suitable primers for amplifying V regions.

In addition to this, the reagent kit can also contain a recipient cell which is suitable for the TCR expression, in particular a human T cell as previously explained.

The application is clarified further by the following figures, sequence listings and examples.

Fig. 1: shows the preparation of the TCRα expression cassette. The primers 5'CαEXPs and 3'CαEXP were used to prepare an amplificate of the TCRα C region, which extended from the third codon to the stop codon, from a TCRα-specific cDNA. The oligonucleotide 5'CαEXPs added recognition sequences for the XmaI and BamHI restriction endonucleases to the 5' ends of the amplified DNA molecules while the oligonucleotide 3'CαEXP added a cleavage site for the SalI restriction endonuclease to the 3' ends of the molecules. The DNA strands were cloned into pBSCIISK+ by way of the XmaI and SalI cleavage sites. After sequencing, an error-free subclone was selected and the Cα region was recloned into the expression vector pSBCII by way of the XmaI and SalI cleavage sites. The vector pSBCII contains two unique restriction cleavage sites, i.e. AseI and NotI, which enable it to be fused with the vector pSBCI (AmpR = ampicillin resistance

gene, SV40P/E = SV40 promoter and enhancer sequences, SV40pA = SV40 polyadenylation signal).

Fig. 2: shows the cloning of the Vα20 domain of the T cell clone 26/B into the TCRα expression cassette. RNA from the RCC-specific clone 26/B was reverse transcribed specifically for the TCR and the resulting cDNA was amplified using the oligonucleotides TCRAV20EXP and 5'CαEXPas. The amplificate comprised the entire Vα20 region (beginning at the start codon) and the first nine codons of the Cα region. The oligonucleotide TCRAV20EXP added an EcoRI cleavage site, and the optimal Kozak sequence, to the 5' end of the amplified DNA molecules while the oligonucleotide 5'-CαEXPas added a BamHI cleavage site to the 3' end of the molecules. Cloning of the DNA molecules into the vector pBSCIISK+ took place by way of the EcoRI and BamHI cleavage sites. After sequencing, an error-free subclone was selected and the Vα20 domain was cloned into the TCRα expression cassette (in pSBCII) by way of the EcoRI and BamHI sites.

Fig. 3: shows the preparation of the TCRβ expression cassette. The primers 5'CβEXPs and 3'CβEXP were used to prepare an amplificate of the TCRβ C region, which extended from the 26th codon to the stop codon, from a TCRβ-specific cDNA. The oligonucleotide 5'CβEXPs added recognition sequences for the SalI and SpeI restriction endonucleases to the 5' ends of the amplified DNA molecules while the oligonucleotide 3'CβEXP added a cleavage site for the HindIII restriction endonuclease to the 3' ends of the molecules. The DNA strands were cloned into pBSCIISK+ by way of the SalI and HindIII

cleavage sites. After sequencing, an error-free subclone was selected and the Cβ region was recloned into the expression vector pSBCI by way of the SalI and HindIII cleavage sites. The vector pSBCI contains two unique restriction cleavage sites, i.e. AseI and NotI, which enable it to be fused with the vector pSBCII, and, in addition, an IRES (internal ribosomal entry site) element.

Fig. 4: shows the cloning of the Vβ22 domain of T cell clone 26/B into the TCRβ expression cassette. RNA from the RCC-specific clone 26/B was reverse transcribed in a manner specific for the TCR and the resulting cDNA was amplified using the oligonucleotides TCRBV22EXP and 5'CβEXPas. The amplificate encompassed the entire Vβ22 region. The oligonucleotide TCRBV22EXP added an EcoRI cleavage site, and the optimal Kozak sequence, to the 5' end of the amplified DNA molecules while the oligonucleotide 5'CβEXPas added a SpeI cleavage site to the 3' end of the molecules. The DNA molecules were cloned into vector pBSCIISK+ by way of the EcoRI and SpeI cleavage sites. After sequencing, an error-free subclone was selected and the Vβ22 domain was cloned into the TCRβ expression cassette (in pSBCI) by way of the EcoRI and SpeI cleavage sites.

Fig. 5: shows the fusion of vectors pSBCI and pSBCII to form the bicistronic TCR expression unit. Vector pSBCI, which contained the TCRβ chain of clone 26/B, and vector pSBCII, which contained the TCRα chain of clone 26/B, were digested with the restriction enzymes AseI and NotI. The two halves, which each encoded a TCR chain, were then ligated again by way of the same cleavage sites such that both TCR chains of

clone 26/B were encoded on the fused expression vector pSBCI/II. The position of the NotI cleavage site led to the IRES element of vector pSBCI coming to lie, in the fusion vector, between the two TCR genes; the intention of this was to enable the Vα chain to be translated in a capping-independent manner (MCS = multiple cloning site).

Fig. 6: shows a TCRα expression cassette. The cDNA sequence in the 5′ region of the Cα region is shown in the middle, with the triplets corresponding to the actual reading frame. The triplets printed in bold correspond to codons 5 and 6 in exon 1. The primer 5′CαEXPs (drawn in above the cDNA sequence) introduces an XmaI cleavage site, and also a BamHI cleavage site, using the degenerate code at position 3 in codon 5 (T to G transversion) and position 3 in codon 6 (C to T transition) (shown at the top) into the 5′ region of the Cα amplificate. The nucleotide substitutions, which only elicit silent mutations in these two codons, and the corresponding amino acids (proline and aspartic acid) are shown at the lower edge of the display. At the same positions, the 3′ regions of the Vα amplificates are modified in an identical manner by the 5′CαEXPas primer (shown below the cDNA sequence), and a BamHI recognition sequence is consequently created once again. A hypothetical primer, which carries a BglII recognition sequence and can inactivate the BamHI site when the Vα segment is cloned, as described in the text, is depicted below the 5′CαEXPas sequence.

Fig. 7: shows the TCRβ expression cassette. The cDNA sequence in the 5′ region of the Cβ region is shown in the middle, with the triplets

corresponding to the actual reading frame. The triplets which are printed in bold correspond to codons 28 and 29 in exon 1. The primer 5'CβEXPs (drawn in above the cDNA sequence) introduces a SalI cleavage site, and also a SpeI cleavage site using the degenerate code at position 3 in codon 29 (G to A transition) (shown at the top), into the 5' region of the Cβ amplificate. The nucleotide substitutions, which only elicit silent mutations in codons 28 and 29, and the corresponding amino acids (threonine and aspartic acid) are shown at the lower edge of the display. At the same positions, the 3' regions of the Vβ amplificates are modified in an identical manner by the 5'CβEXPas primers (shown below the cDNA sequence), and an SpeI recognition sequence is consequently created once again. A hypothetical primer, which carries an XbaI recognition sequence and can inactivate the SpeI site when cloning the Vβ segment, as described in the text, is depicted below the 5'CβEXPas sequence. The places at which oligonucleotides 5'CβEXPs and 5'CβEXPas hybridize are identical in Cβ1 and Cβ2.

Fig. 8: shows a model of the "synthetic" T cell. The cell line Jurkat 9-5 (center) carries the gene for β-galactosidase (lacZ), which gene is under the control of the IL-2 promoter and is stably integrated into the genome. Successful transfection of this cell with the pSBCI/II fusion vector leads to the TCR chains which are encoded by it being expressed on the cell surface. Interaction of the expressed TCR with its specific ligand leads to the IL-2 promoter being activated and consequently to the intracellular production of β-galactosidase. When the Jurkat cells are lyzed, the

β-galactosidase is released and can convert a substrate, resulting in the reaction solution becoming colored.

Fig. 9 and
SEQ ID NO. 1 to 8: Oligonucleotides for the TCR expression cassettes

Examples

Example 1 Preparing a bicistronic TCR expression unit

The TCR of the RCC-specific cytotoxic clone 26/B was selected for preparing a TCR expression system. The α chain gene of this TCR consists of the variable region Vα20 and the constant region Cα. The TCRβ chain gene consists of the variable region Vβ22 and the constant region Cβ. The sequences of these, and all the other known V gene segments, are published in Arden et al. (Immunogenetics 42 (1995), 455-500).

As explained in Figures 1 to 4, the TCR chains were cloned in two steps: in the first step, the C regions were cloned (Figures 1 and 3), i.e. the actual cassettes were completed, and, in the second step, the corresponding V regions were inserted into the expression cassettes (Figures 2 and 4). The basic vectors employed were pSBCI and pSBCII (Dirks et al., Gene 128 (1993), 247-249). These vectors are low copy plasmids and for this reason all the fragments were subcloned in pBSCIISK+ (from Stratagene, Catalog No. 21 22 05). This vector replicates to a high copy number in bacteria and in addition proved to be particularly compatible with the pBSC vectors as far as the restriction cleavage sites were concerned.

The fragments contained in pBSCIISK+ were sequenced and the DNA from error-free subclones was recloned into the expression vectors. Primers which inserted various

modifications into the corresponding DNA regions were employed for amplifying the TCR-specific cDNA. The 5′ primers for amplifying the C region (5′CαEXPs and 5′CβEXPs, cf. Fig. 9 and SEQ ID NO. 3 and 7) in each case carried two recognition sequences for restriction endonucleases, with the first of these recognition sequences being freely selectable and being used to clone the cassette into the expression vector. The second recognition sequence constituted the actual cassette cleavage site and enabled the V region to be cloned in subsequently (see below). The 3′ primers employed were the oligonucleotides 3′CαEXP and 3′CβEXP (Fig. 9 and SEQ ID NO. 4 and 8). The coding segment of the respective C gene segment, including the stop codon, was amplified by a PCR (protocol: predenaturation 94°C, 2 min, 1 cycle; denaturation 94°C, 30 sec, 30 cycles; annealing 63°C, 30 sec, 30 cycles; extension 72°C, 1 min, 30 cycles and final polymerization 72°C, 10 min, 1 cycle) using the relevant α-β primer combination. A cleavage site for cloning was also inserted in each case (Figs. 1 and 3).

The primers TCRAV20EXP and TCRBV22EXP (Fig. 9 and SEQ ID NO. 1 and 5), which were specific for the V gene segment, were used for cloning the V regions of clone 26/B. These primers bind to the V gene sequence in the region of the start codon and at the same time encode the optimal Kozak sequence and a restriction cleavage site. The Kozak sequence (CCRCCAUG (G), R = A or G) describes the optimal sequence environment for the initiation codon AUG, which environment is important for translation to be initiated efficiently at this start codon. The 3′ primers, 5′CαEXPas and 5′CβEXPas (Fig. 9, SEQ ID NO. 2 and 6) hybridize in the 5′ region of the respective C gene segment and introduced mutations corresponding to the cassette cleavage sites which were in each case employed (Figs. 2 and 4).

The bicistronic expression unit was prepared by fusing the two vectors pSBCI and pSBCII with the aid of two unique restriction cleavage sites (AseI and NotI, Fig. 5). In the resulting fusion vector pSBCI/II, the two TCR chains flank an IRES element, which leads to capping-independent translation initiation and consequently enables both TCR protein chains to be synthesized efficiently. Since the capping-dependent translation of the gene which is located 5' of the IRES element functions somewhat more efficiently than does that of the gene which is located 3', the TCRβ chain was cloned into pSBCI and the TCRα chain was cloned into pSBCII. This thereby limited expression of the α chain as compared with expression of the β chain, with the aim of preventing the formation of TCRα homodimers.

As has already been mentioned above and depicted in Fig. 5, SfiI cleavage sites were incorporated upstream of the TCRβ chain and downstream of the TCRα chain in order to enable the two chains to be recloned together with the IRES element located between them. The same SfiI cleavage site is also present in the multiple cloning site of the vector pHEBNA-1 (Mautner et al., Oncogene 12 (1996), 1299-1307 and Mucke et al., Gene Ther. 4 (1997), 82-92), which is able to replicate episomally and therefore renders stable transfection unnecessary.

Example 2 Restriction cleavage sites in the expression cassette

The restriction cleavage sites in the expression cassettes, which sites enable any TCR V domains to be cloned upstream of already completed C domains with no change in the TCR amino acid sequence, are a central point in the preparation of "synthetic T cells". Figs. 6 and 7 provide a diagrammatic representation of the construction and mode of function of the TCRα and β expression cassettes.

In particular, the diagrams depict the 5' region of the respective C gene segments in which the amplificates of the V region and C region overlap. As can be seen in detail in the figures, the listed restriction cleavage sites were inserted by means of mutations in the oligonucleotides employed. In this connection, the first cleavage site in each case was used for cloning the C gene segment into the expression vectors (TCRα:XmaI, TCRβ:SalI). The V gene segments were cloned in by means of the second cleavage site in each case (TCRα:BamHI, TCRβ:SpeI). Since the first cleavage sites were removed by cloning in the V gene segments, no consideration had to be given to reading frame shifts and/or amino acid substitutions when selecting them.

When the regions to be cloned were examined, endogenous restriction cleavage sites for XmaI and BamHI were found to be present in the coding region of Vα20 and in the 3'-untranslated region of Cα, respectively. Since, however, the V and C segments were cloned independently of each other, and, in addition to that, the C regions were only cloned up to the start codon, it was possible to ignore the two endogenous recognition sequences. As previously mentioned, restriction cleavage sites in the expression cassette can be inactivated if required, something which is necessary when a Vα domain possesses an endogenous BamHI cleavage site or a Vβ domain possesses an endogenous SpeI cleavage site. In order to do this, the 3' primers which are used for amplifying the V region can be used to insert any restriction cleavage sites whose core sequences, i.e. the central four bases of the recognition sequence, are identical to the corresponding sequences of the BamHI or SpeI cleavage site, respectively (Table 1).

Table 1

TCR cassette	Compatible restriction endonucleases	Can be recut with
α: BamHI (=BstI)	BclI, BglII, BstYI (= MflI, XhoII), MboI (= DpnII, Sau3AI)	BstYI, MboI
β: SpeI	AvrII, NheI, StyI, XbaI	BfaI

The restriction endonucleases which are listed in the second column of Table 1 generate cohesive ends which are partially identical to the restriction cleavage sites of the expression cassette which are given in the first column. While these enzymes can be used to inactivate the cassettes with respect to the original enzymes, the cassettes can be recut by the enzymes which are listed in the third column. The enzymes which are listed in brackets are isoschizomers.

In Figs. 6 and 7, BglII and XbaI are given as examples of restriction enzymes listed in the second column of Table 1 which are compatible in the case of the TCRα chain and the TCRβ chain, respectively. In this connection, it is important that the enzymes employed cut asymmetrically. The inactivation is based on the two "incorrect" nucleotides having no effect on the completed polypeptide chains. In the case of both the TCR chains, the first nucleotide does not result in any amino acid substitution since all the four possible bases at these positions encode identical amino acids (Figs. 6 and 7). Due to the restriction endonucleases cutting asymmetrically, the second incorrect nucleotide is incorporated into the opposing strand and does not therefore have any effect on the translated mRNA.

Example 3 Expression of the T cell receptor in eukaryotic cells

In order to make it unnecessary to transfect additional accessory molecules such as CD3, CD4 and/or CD8, the cloned TCR is preferably expressed in a human T cell.

Essentially two aspects were of prime importance in selecting the recipient cells. On the one hand, the aim was for the recipient cell to have a high potential for proliferation, both in order to have available a sufficiently large number of cells for the transfection experiments in a relatively short time and to be able to expand successfully transfected clones substantially even after a large loss in cell number due to the transfection as such. In addition to this, the aim was for the recipient cell to exert an unambiguous and measurable biological function after having been specifically stimulated by way of the transfected TCR.

Various human T cell clones and T cell lines which are in principle suitable for use as recipient cells on the basis of their growth and other properties are described briefly below.

The human T cell clone 234 is an example of a suitable recipient cell. This clone possesses some special properties: a high growth potential, cytotoxicity and an endogenous receptor which does not recognize any determinants on RCC-26 tumor cells. This non-recognition is particularly important since this clone is not a transformed cell which therefore needs to be regularly restimulated by way of its endogenous TCR. The greatest advantage lies in the cytotoxicity of the cell, which cytotoxicity can be measured readily and unambiguously as a demonstration of specific T cell activation.

Other recipient cells which come into consideration are established human cell lines such as Molt-4, Peer, Jurkat and different Jurkat variants. These lines offer the advantage of rapid growth and can also be transfected with a high degree of efficiency. However, a disadvantage for certain applications is that they are transformed cell lines which are unsuitable for therapeutic use and do not possess any cytotoxicity.

Specific activation of these T cells can be demonstrated with the aid of secreted cytokines such as interleukin 2 (IL-2) and tumor necrosis factor (TNF).

Activation can be demonstrated particularly readily using a special variant of the Jurkat cell (Jurkat 9-5) which carries the β-galactosidase gene (LacZ) stably integrated into the genome and under the control of the IL-2 promoter. As shown in Figure 8, stimulation of the transfectant by way of its endogenous or transfected TCRs leads to activation of the IL-2 promoter and consequently to transcription of the LacZ gene and accumulation of β-galactosidase in the cytosol. The addition of a buffer which contains a detergent and a substrate for the β-galactosidase results in the cells being lyzed, and the presence of the indicator enzyme can then be demonstrated by the reaction buffer becoming colored.

SEQUENCE LISTING

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Claims

1. A basic vector for preparing a TCR expression vector, which possesses an expression control sequence operatively linked to a polycistronic expression unit comprising:
   (a) at least a part of the nucleotide sequence encoding a C region of the TCRα chain, with at least one restriction cleavage site being located in the 5′ region of the nucleotide sequence, and
   (b) at least a part of the nucleotide sequence encoding a C region of the TCRβ chain, with at least one restriction cleavage site being located in the 5′ region of the nucleotide sequence.

2. A vector as claimed in claim 1, characterized in that it can be propagated in eukaryotic cells.

3. A vector as claimed in claim 1 or 2, characterized in that it is a plasmid.

4. A vector as claimed in one of the preceding claims, characterized in that it is a vector which can be replicated episomally.

5. A vector as claimed in one of the preceding claims, characterized in that the expression unit additionally contains a sequence which allows the polycistronic transcription product to be translated in a capping-independent manner.

6. A vector as claimed in claim 5, characterized in that the expression unit contains an IRES sequence.

7. A vector as claimed in one of the preceding claims, characterized in that the expression unit

allows an expression of the TCRα chain which is limited as compared with expression of the TCRβ chain.

8. A vector as claimed in one of the preceding claims, characterized in that the C regions of the TCRα and TCRβ chains are of human origin.

9. A vector as claimed in one of the preceding claims, characterized in that at least one of the restriction cleavage sites located in the 5′ region of the nucleotide sequences encoding the C regions of the TCRα and TCRβ chains is unique.

10. A vector as claimed in one of the preceding claims, characterized in that the restriction cleavage sites do not result in any amino acid substitution in the C regions.

11. A vector as claimed in one of the preceding claims, characterized in that a BamHI cleavage site and/or an XmaI cleavage site is/are located in the 5′ region of the DNA sequence encoding the C region of the TCRα chain.

12. A vector as claimed in one of the preceding claims, characterized in that a SpeI cleavage site and/or a SalI cleavage site is/are located in the 5′ region of the DNA sequence encoding the C region of the TCRβ chain.

13. A TCR expression vector, which possesses an expression control sequence operatively linked to a polycistronic expression unit comprising:

(a) a nucleotide sequence encoding a complete TCRα chain and

(b) a nucleotide sequence encoding a complete TCRβ chain,

with the nucleotide sequences encoding the V regions and C regions of the TCR chains being linked to each other by way of restriction cleavage sites in the 5′ region of the C regions.

14. A TCR expression vector as claimed in claim 13, characterized in that the nucleotide sequences encoding the TCR chains possess at least one base substitution, as compared with the natural TCR sequence, in the region of the restriction cleavage sites, with the base substitutions being selected within the context of the degeneracy of the genetic code.

15. A process for preparing a TCR expression vector as claimed in claim 13 or 14, comprising the steps of:

(a) preparing a basic vector as claimed in one of claims 1 to 12, and

(b) inserting nucleotide sequences which contain the regions encoding a desired V region of a TCRα or TCRβ chain into the restriction cleavage sites which are located in the 5′ regions of the nucleotide sequences encoding the C regions, or

(a′) preparing two vectors, each of which contains at least a part of the nucleotide sequence encoding the C region of the TCRα chain or the TCRβ chain, respectively, with at least one restriction cleavage site being located in the 5′ region of the nucleotide sequence,

(b′) inserting nucleotide sequences which contain the regions encoding a desired V region of a TCRα chain or the TCRβ chain into the restriction cleavage sites which are located in the 5′ regions of the nucleotide sequences encoding the C regions, and

(c') assembling the nucleotide sequences encoding the TCR chains in order to obtain a TCR expression vector.

16. A cell, characterized in that it is transformed with a basic vector as claimed in one of claims 1 to 13.

17. A cell, characterized in that it is transformed with a TCR expression vector as claimed in one of claims 13 to 14.

18. A cell as claimed in claim 17, characterized in that it is a mammalian cell.

19. A cell as claimed in claim 17 or 18, characterized in that it is able to express one or more accessory molecules.

20. A cell as claimed in one of claims 17 to 19, characterized in that the accessory molecules are selected from the group consisting of CD3, CD4, CD8 and cytokines such as IL-2 and/or TNF.

21. A cell as claimed in one of claims 17 to 20, characterized in that it is a human T cell.

22. A cell as claimed in one of claims 17 to 21, characterized in that it is selected from the T cell clones and T cell lines 234, molt-4, Peer and Jurkat and variants thereof.

23. A process for expressing a T cell receptor, characterized in that a suitable host cell is transformed with an expression vector as claimed in claim 13 or 14 and the cell is cultured under conditions which lead to the T cell receptor being expressed.

24. A reagent kit for preparing a TCR expression vector, comprising
    (a) a basic vector as claimed in one of claims 1 to 12, and
    (b) primers for amplifying V regions of the TCRα and TCRβ chain genes.

25. A reagent kit for preparing a TCR expression vector, comprising
    (a) two separate vectors containing the elements of the expression unit from a basic vector as claimed in one of claims 1 to 12, and
    (b) primers for amplifying V regions of the TCRα and TCRβ chain genes.

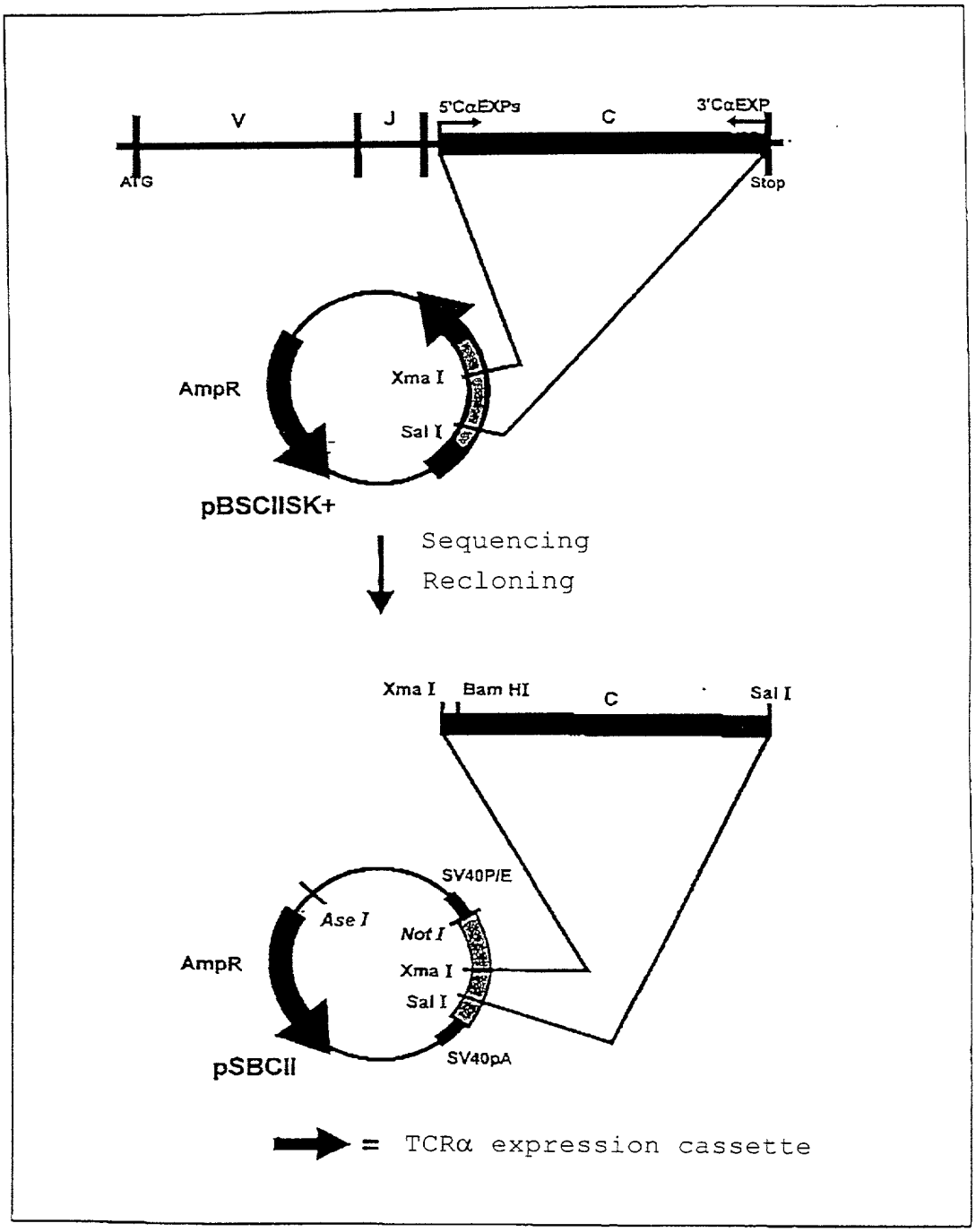
26. A reagent kit as claimed in claim 24 or 25, which additionally comprises a recipient cell which is suitable for TCR expression.

Abstract

The invention relates to a process for expressing T cell receptors and to vectors which are suitable for this. In addition, this present document discloses cells which are transfected with the vectors and which are able to express T cell receptors which are in each case desired.

vo 04.06.98 12:37

Figure 1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Sequencing
Recloning
Xma I
Bam HI
C
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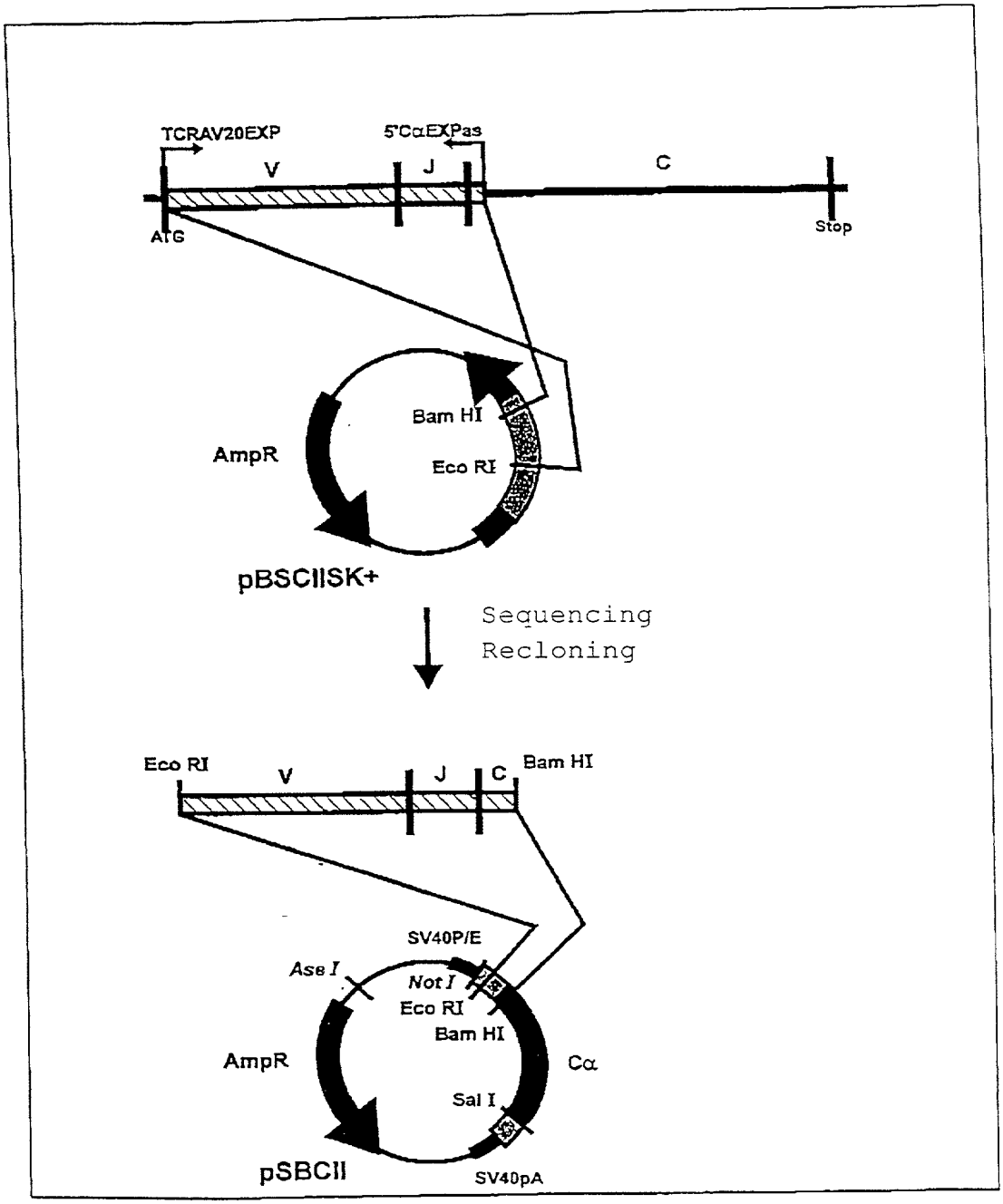
pSBCII
SV40pA
= TCRα expression cassette

Figure 2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SV40pA

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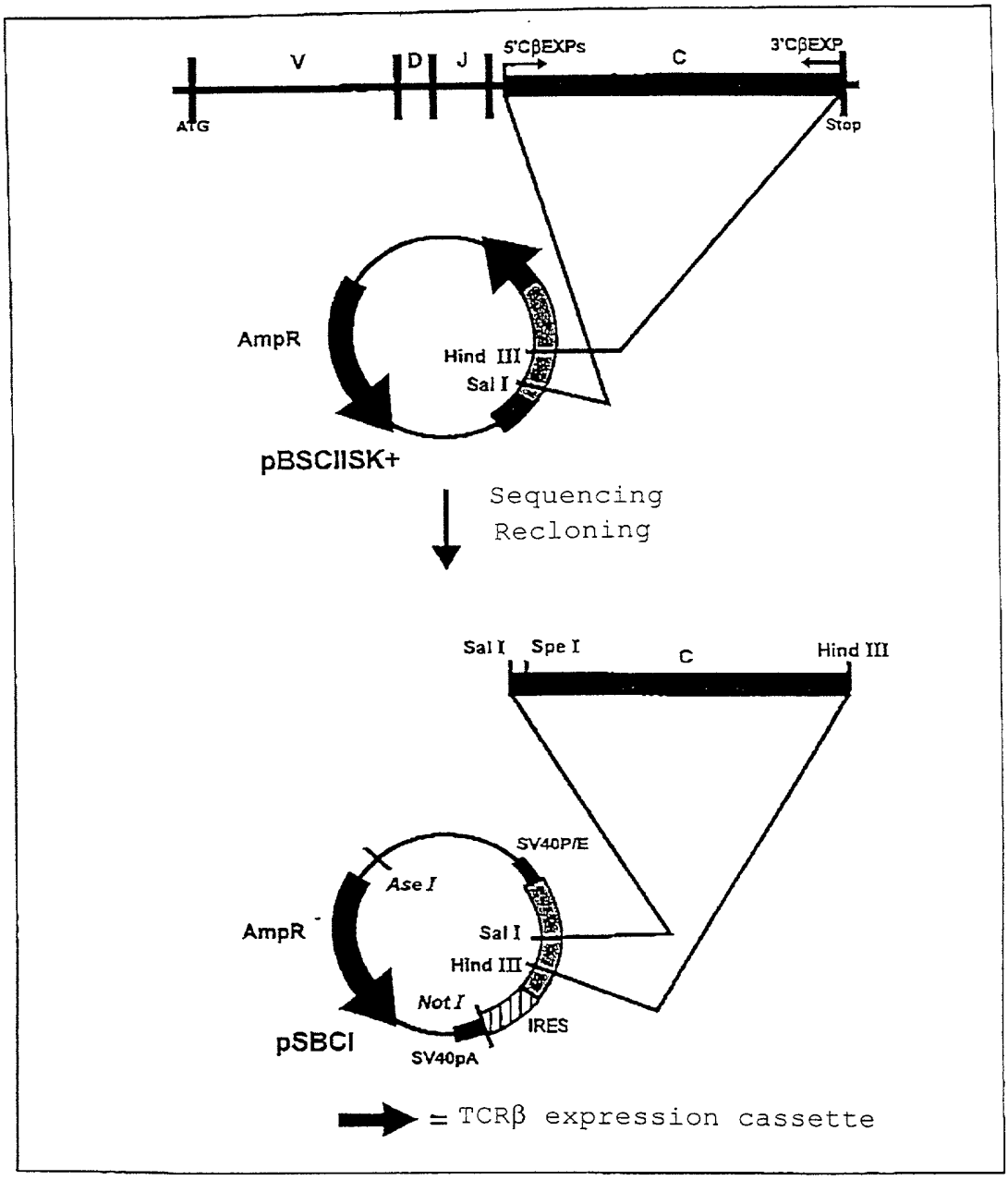
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Spe I
C
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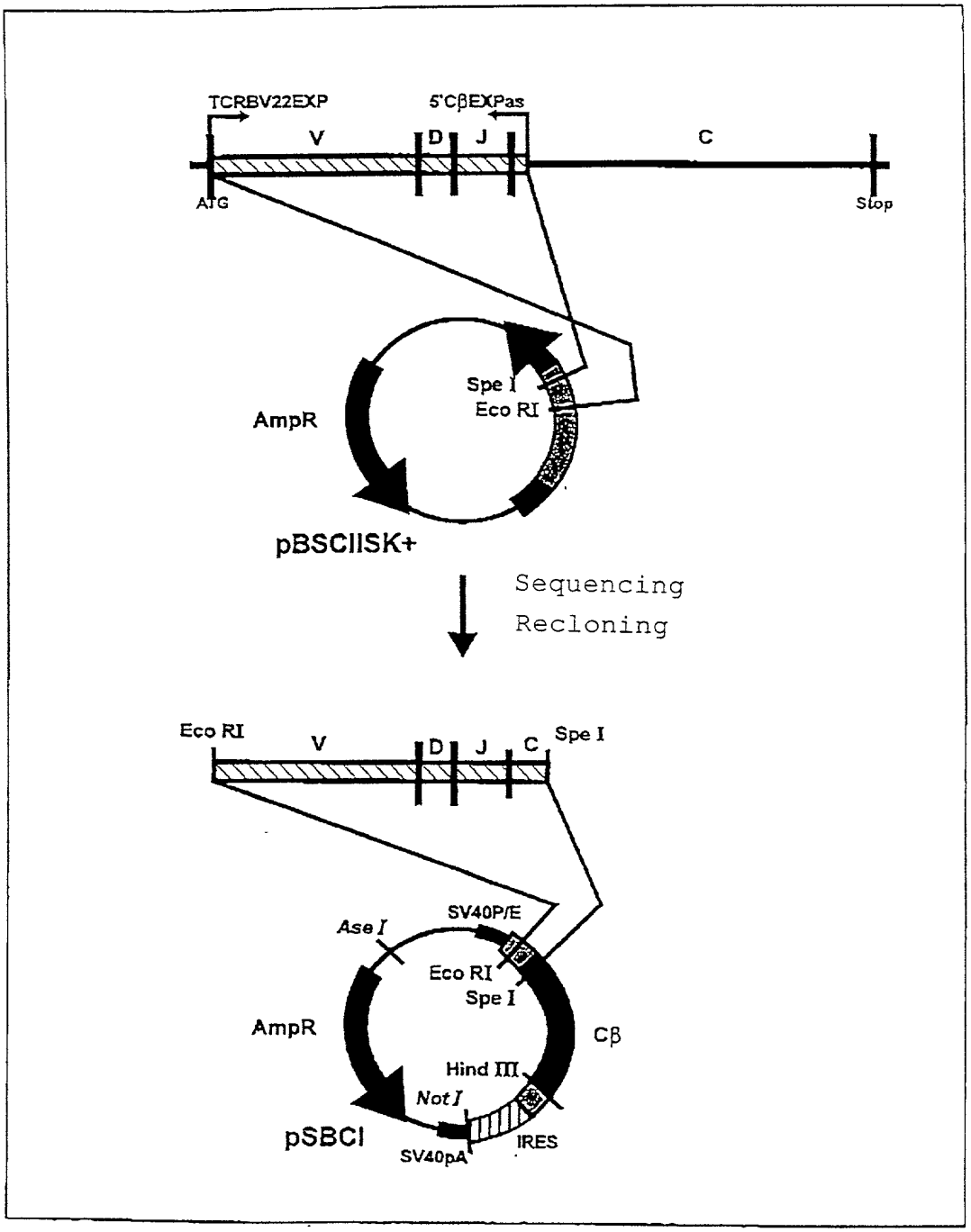
pSBCI
SV40pA
= TCRβ expression cassette

Figure 4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D
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C
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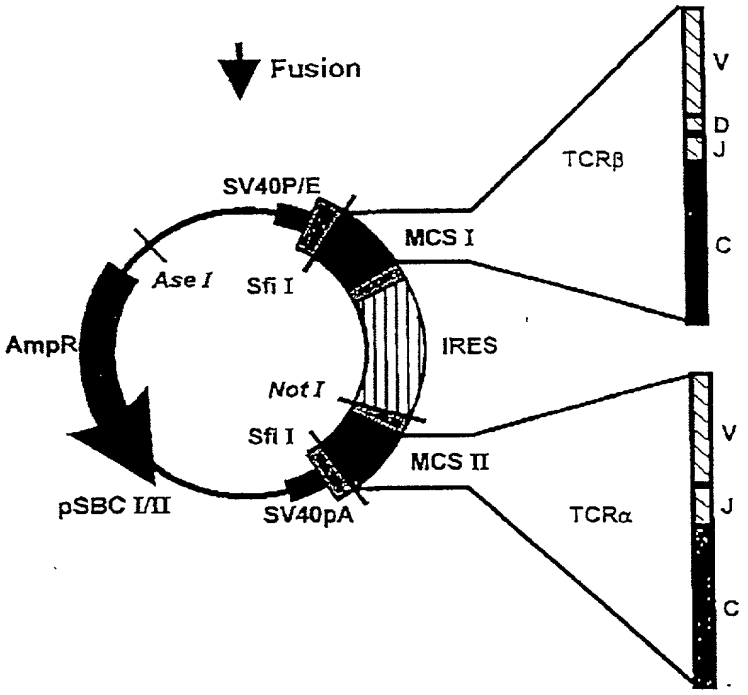
IRES

Figure 5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V
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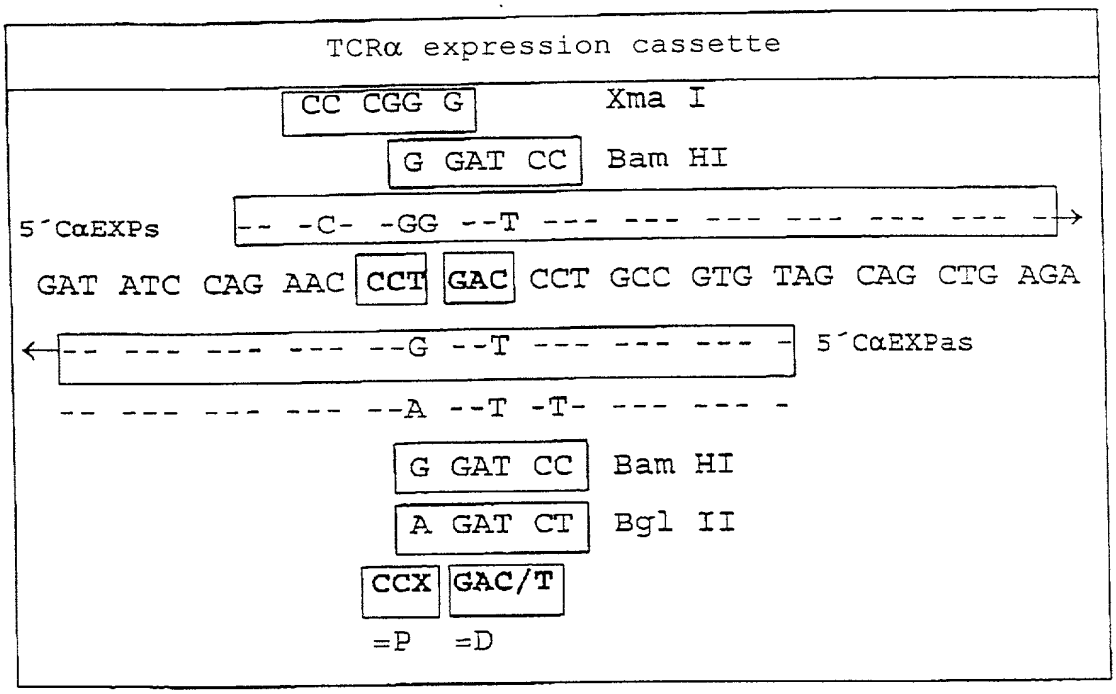
Figure 6



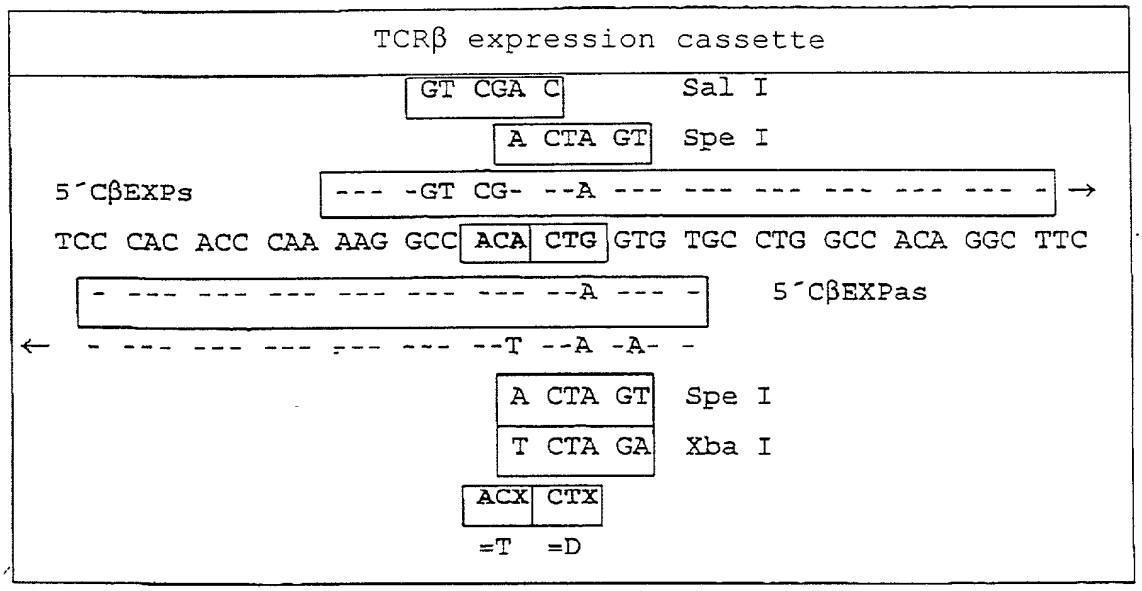


Figure 7

Figure 8

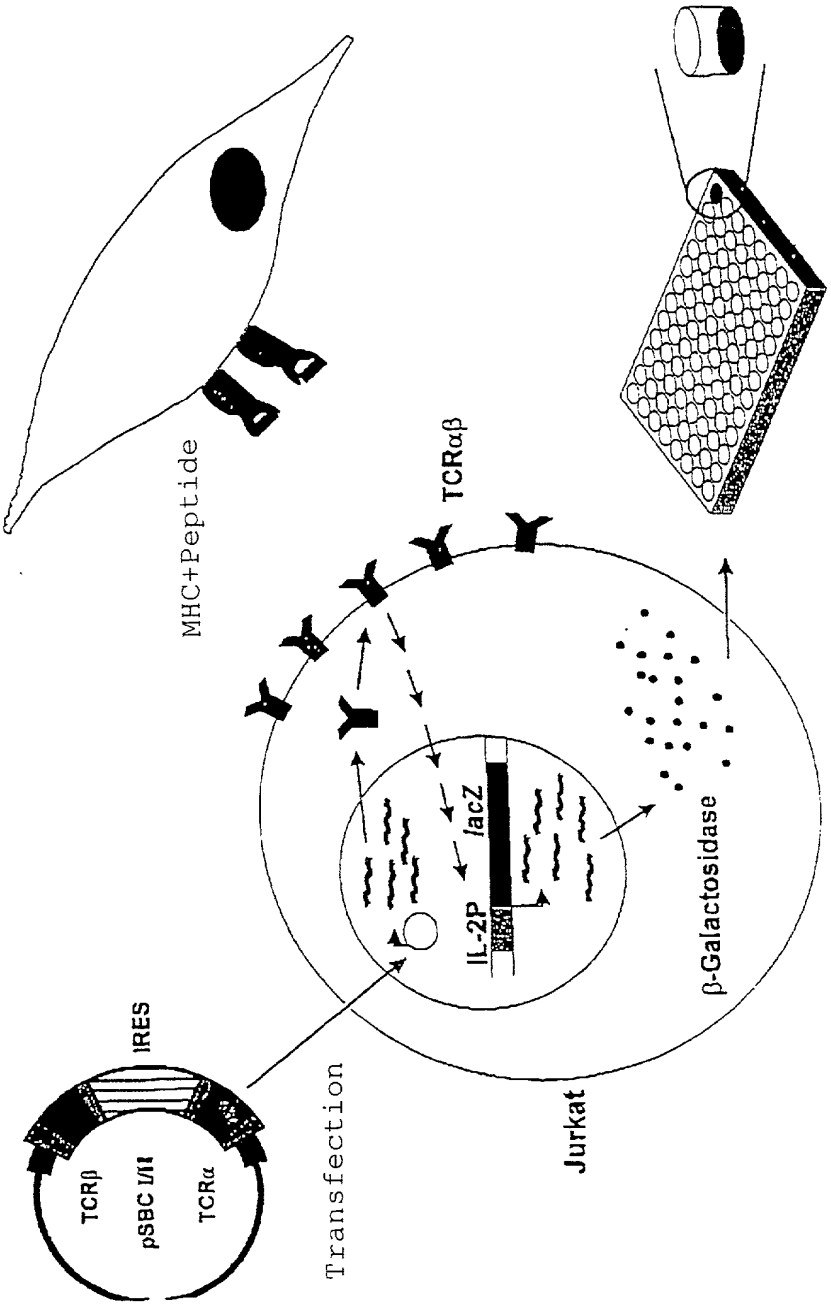

MHC+Peptide
TCRαβ
IRES
TCRβ
pSBC I/II
TCRα
Transfection
IL-2P
lacZ
β-Galactosidase
Jurkat

Figure 9

Oligonucleotides for the TCR expression cassettes

Oligonucleotides for the TCRAV20PCR

TCRAV20EXP

5' CCGAATTC***CCACCATG***AGGCAAGTGGCGAGAGTA 3'
Eco RI — Kozak

5'CαEXPas

5' ACACGGCAGGATCCGGGTTCTGGATAT 3'
Bam HI

Oligonucleotides for the TCRACPCR

5'CαEXPs

5' AGA***CCCGGG***ATCCTGCCGTGTACCAGCTGA 3'
Xma I Bam HI

3'CαEXP

5' TCCCGTCGAC***GGCCTCACTGGCC***TCAGCTGGACCACAGCCG 3'
Sal I Sfi I Stop

Oligonucleotides for the TCRBV22PCR

TCRBV22EXP

5' CCGAATTC***GGCCTCACTGGCC***ACCATGGATACCTGGCTCGTA 3'
Eco RI Sfi I Kozak

5'CβEXPas

5' ACACTAGTGTGGCCTTTTGGGTGT 3'
Spe I

Oligonucleotides for the TCRBCPCR

5'CβEXPs

5' AAGGGTCG***ACTAGT***GTGCCTGGCCACAGGCT 3'
Sal I Spe I

3'CβEXP

5' GTTAAGCTT***CTA***GCCTCTGGAATCCTT 3'
Hind III Stop

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Docket No. _____

ARENT FOX KINTNER PLOTKIN & KAHN, PLLC
Nikaido, Marmelstein, Murray & Oram Intellectual Property Group

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention **entitled**
(Insert Title) T cell receptor expression cassette

the specification of which is attached hereto unless the following box is checked:

XX was filed on March 30, 1999 as PCT International Application
Number PCT/EP 99/02171 and was amended on _____

and/or was filed on _____ as United States Application
Number _____ and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.
I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56.
I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International Application having a filing date before that of the application(s) for which priority is claimed:

(List prior foreign applications. See note A on back of this page)	(Number)	(Country)	(Day/Month/Year Filed)	Priority Claimed
	198 16 129.8	DE	April 09, 1998	☒ Yes ☐ No
				☐ Yes ☐ No
				☐ Yes ☐ No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)

(See Note B on back of this page) ☐ See attached list for additional prior foreign or provisional applications.

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) (U.S. or PCT) in the manner provided by the first paragraph of 35, U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(List prior U.S. Applications or PCT International applications designating the U.S.)	(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

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And I hereby appoint as principal attorneys: Robert B. Murray, Reg. No. 22,980; Charles M. Marmelstein, Reg. No. 25,895; George E. Oram, Jr., Reg. No. 27,931; Douglas H. Goldhush, Reg. No. ~~33,125~~; David T. Nikaido, Reg. No. 22,663; Monica Chin Kitts, Reg. No. 36,105; Richard J. Berman, Reg. No. 39,107; King L. Wong, Reg. No. 37,500; James A. Poulos, III, Reg. No. 31,714; Patrick D. Muir, Reg. No. 37,403; Murat Ozgu, Reg. No. 44,275; Bradley D. Goldizen, Reg. No. ~~43,637~~, N. Alexander Nolte, Reg. No. ~~45,689~~ and Robert K. Carpenter, Reg. No. 34,794.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(See Note C on back of this page)

Full name of sole or first inventor Schendel Dolores
Inventor's signature Dolores J. Schendel Date
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Full name of third joint inventor, if any _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____

Full name of fourth joint inventor, if any _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____

Full name of fifth joint inventor, if any _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____

Full name of sixth joint inventor, if any _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____

Full name of seventh joint inventor, if any _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____

Full name of eighth joint inventor, if any _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____

Full name of ninth joint inventor, if any _____

Inventor's signature _____ Date

Residence _____

Citizenship _____

Post Office Address _____